Journal of Medicinal and Chemical Sciences 6 (2023) 2096-2103

Journal of Medicinal and Chemical Sciences

Journal homepage: http://www.jmchemsci.com/

*Original Article*

# Effectiveness Comparison of Decontamination Sputum Specimen between Kudoh and Petroff Modification Technique in *Mycobacterium Culture*

**Andritta Febriana[1], Ni Made Mertaniasih[2,3]*, Pepy Dwi Endraswari[2,3], Lindawati Alimsardjono[2,3], Eko Budi Koendhori[2,3]**

[1]*Study Program of Clinical Microbiology Specialist, Faculty of Medicine, Airlangga University*
[2]*Department of Medical Microbiology, Faculty of Medicine, Airlangga University, Surabaya, Indonesia*
[3]*Department of Clinical Microbiology, Faculty of Medicine, Airlangga University, Dr. Soetomo Hospital, Jl. Mayjen. Prof. Dr. Moestopo No. 47, Surabaya 60131, Indonesia*

**ARTICLE INFO**

**Article history**

Receive: 2023-01-24
Received in revised: 2023-02-18
Accepted: 2023-03-31
Manuscript ID: JMCS-2301-1939
Checked for Plagiarism: **Yes**
Language Editor:
Dr. Fatimah Ramezani
Editor who approved publication:
Professor Dr. Ali Delpisheh

**DOI:10.26655/JMCHEMSCI.2023.9.16**

**KEYWORDS**

Tuberculosis
Decontamination
Kudoh
Modified Petroff

**ABSTRACT**

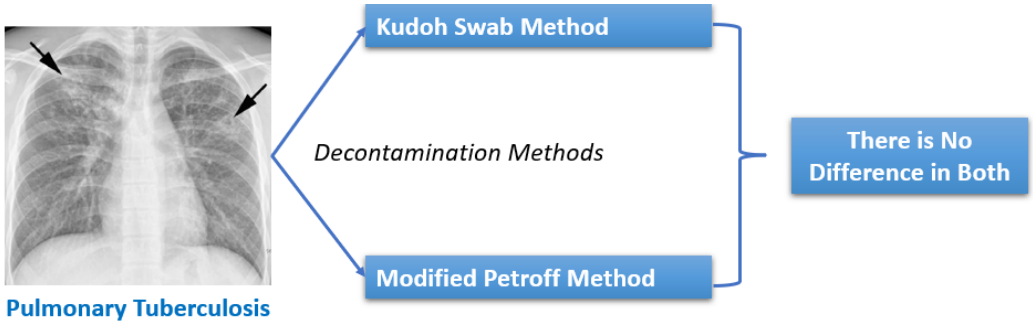
**Objectives:** Tuberculosis (TB) is the common cause of death due to a single infectious agent. MTB culture is still the gold standard for TB diagnosis. For optimal care of pulmonary TB, effective decontamination methods are required to isolate and identify MTB. The simpler Kudoh decontamination/culture method swab was compared with modified Petroff to isolate mycobacteria from sputum specimens.

**Materials and Methods:** A total of 15 sputum samples were collected from patients with Xpert MTB/RIF assay-confirmed pulmonary TB. The samples were processed with both decontamination methods. Each sample group was inoculated directly on the Lowenstein-Jensen (LJ) and the Middlebrook 7H10 agar medium. The growth of contamination, recovery rate, and detection time were observed in both media.

**Results:** Out of 15 samples, the contamination rate on the LJ by both methods was 2 (13%), respectively, whereas the Middlebrook 7H10 by both methods showed 1 (6.6%). The recovery rate on the LJ was 13 (86.6%) by the Kudoh method and 14 (93.3%) by the modified Petroff method, while 13 (86.6%) showed positive culture on the Middlebrook 7H10 by both. In this study, positive culture results using the modified Petroff technique showed more colony growth than the Kudoh technique, indicating more viable MTB colonies than the Kudoh technique.

**Conclusion:** Petroff modification technique shows the growth of the number of colonies more than Kudoh technique, but the Kudoh technique shows the results of positive culture that is no different to the recovery level even though the number of colonies that grow in less Kudoh technique.

**GRAPHICAL ABSTRAC**




* Corresponding author: Ni Made Mertaniasih
✉ E-mail: nmademertaniasih@gmail.com

## Introduction

Indonesia is a country that has a high burden of tuberculosis (TB). It ranks the 3rd country in absolute numbers (81,137 cases reported in 2015) and the 20th in incidence (41.0/100 thousand inhabitants), considering the list of the 20 countries responsible for approximately 84.0% of the world's burden of TB. The goals proposed by the Global End Tuberculosis Strategy report by the World Health Organization (WHO) are an 80% reduction in incidence rate (new and relapse cases per 100 000 population per year), and a 90% reduction in the annual number of TB deaths by 2020, compared with data from 2015 [1]. Therefore, it is necessary to monitor bacteriological confirmation of TB to establish a diagnosis in a quick and precise manner [2], start the most effective treatment as early as possible, so that good treatment outcomes to be able to limit its transmission and obtain successful TB control [3]. The clinical and radiological picture can provide vital signs of the disease. Still, the definitive diagnosis is reached by verifying the presence of etiologic agent in respiratory samples or those coming from other sites.

Culture remains the gold standard for TB diagnosis [4]. Culture can increase the bacteriologic diagnosis of the disease by up to 30.0% [2] because it can detect at least 10-100 mycobacteria/ml specimen [5]. Cultivation is the only method that enables differentiation between viable and dead bacterial cells, which can frequently recruit to monitor treatment response, confirm patient sterility and sputum conversion, ensure the breaking of transmission cycles, and document the cure of patients [6]. Moreover, it allows further identification of the isolated mycobacterium and susceptibility tests to antimicrobial agents. Culturing clinical samples is crucial for control and prevention strategies with the worldwide increase in drug-resistant TB [4]. Nevertheless, before inoculation, it is necessary to treat the samples of nonsterile sites to eliminate microorganisms of natural and environmental microbiota, favoring the growth and isolation of mycobacteria.

The modified Petroff method is the standard for performing decontamination of the samples. However, this method requires many steps, takes longer to process sputum, and requires centrifugation and a biosafety cabinet, which cannot be feasible in some settings [2]. The modified Petroff method is a standard decontamination method using 4% NaOH. The study conducted by Sotomayor *et al.* regarding the modified Petroff method showed a recovery rate of 96.6% [4]. Another study showed a hazard rate of 12% for this method [7].

Kudoh (1974) introduced a simple decontamination technique using a sterile cotton swab to collect sputum in clinical samples and the decontamination process only takes 3-4 minutes/sample, can be done in laboratories with limited facilities, does not require technical skills, biosafety cabinet (BSC) and centrifugation which can generate infectious aerosols. MTB present in sputum can be concentrated onto the cotton swab because the mycobacteria glycolipid cell wall easily adheres to the solid surface of the swab. This method is efficient for detecting MTB even in samples with negative microscopic examination [2].

A study conducted by Madeira *et al.* showed that the Kudoh technique had a sensitivity of 80% and a specificity of 93.5% with a contamination level of 4.1% [8]. Another study showed that Kudoh technique can detect the MTB growth faster than the modified Petroff technique. The contamination rate for the two techniques did not show any difference. The short duration of exposure to 4% NaOH solution using the Kudoh technique is expected to kill contaminating microorganisms and maintain the viability of MTB [9]. This study aims to prove the effectiveness of the Kudoh decontamination technique to reduce contamination and maintain recovery in MTB cultures.

## Materials and Methods

*Study design, sample collection, and sample size*

This experimental laboratory study was carried out at the Microbiology Clinic Department of Soetomo Hospital, Surabaya, Indonesia, after getting approval from the Health Research Ethics

Committee at Soetomo hospital on February 2022. The study sample is all sputum specimens of patients with a diagnosis of pulmonary TB and confirmed by the Xpert MTB/RIF assay that meets the minimum sample size and inclusion criteria. The inclusion criteria for sample acceptance were sputum arriving until the GeneXpert process was less than 48 hours, stored at 2-8 ºC, a volume of 5 mL, and mucoid consistency. Totally, 15 sputum samples were collected.

*Specimens processing*

Samples were first processed by the previously described Kudoh-swab method, and then processed using modified Petroff. Following the World Health Organization (WHO) recommendations, sputum samples were mL of sputum was transferred to a Falcon type-tube, and an equal volume of 4% NaOH solution was added into the tube. The mixture was homogenized and placed for 15 minutes at room temperature for fluidification-decontamination. The falcon tube was tightly closed and vortexed until homogeneous and allowed to stand for no more than 15 minutes at room temperature. After decontamination, the samples were neutralized with phosphate-buffered saline (PBS; 0.067 M) by adding PBS solution to the Falcon tube until the volume was > 45 mL and centrifuged for 15 min at 3000× g. After centrifugation, the Falcon tube was allowed to stand for 10 minutes so that the aerosol fell. The supernatant was discharged into a container containing the disinfectant. 1-3 mL of PBS solution was added to the sediment and shaken until homogeneous. Each 200 µl of the sediment was inoculated in each LJ and Middlebrook 7H10 medium and subjected to AFB smear microscopy.

*Analysis of culture result*

Inoculated media were incubated at 37 °C, and mycobacterial growth was evaluated weekly for up to 8 weeks. (The observation procedure for colony growth is carried out every week, with the average time detected by growth between 3 and 4 weeks). Culture results reported positive if processed and analyzed by direct Ziehl-Neelsen microscopy [10].

*Kudoh swab method*

Sputum samples were impregnated in cotton swabs [11], taken at the most purulent part [2], and then immersed consecutively in a tube containing 2 ml of 4% NaOH solution for decontamination. After 2 min, swabs were removed from the test tube. Thereafter, each swab was inoculated in the LJ and the Middlebrook 7H10 medium with rotary movements and subjected to AFB smear microscopy [10].

*Modified petroff method*

2

acid-fast colonies grew in the medium as determined with the Ziehl-Neelsen stain technique. MPT64 tests were performed to confirm positive cultures for MTB. After eight weeks, cultures with no mycobacterial growth were considered as negative. AFB-positive cultures were identified using phenotypic tests [10]. Phenotypic tests were used to confirm the MTB isolation. Cultures were considered to be contaminated when microorganisms other than mycobacteria grew on the media with or without media liquefaction [12].

*Statistical analysis*

The Man-Whitney test was used to compare the recovery rate, the contamination rate, and time detection with a p-value <0.05 were considered as statistically significant. The calculations were made using SPSS 25.0 for data processing.

## Results and Discussion

In ZN staining, 2 (13.3%) samples were smeared negative before the decontamination procedure was carried out. After decontamination, the results of ZN staining with the Kudoh and the modified Petroff techniques showed that all specimens (100%) were positive for acid-fast (Table 1).

**Table 1:** ZN staining results

| Process Decontamintaion | Negative n (%) | Scanty n (%) | 1+ n (%) | 2+ n (%) | 3+ n (%) | Total n (%) |
|---|---|---|---|---|---|---|
| Pre-decontamination | 2 (13.3%) | 1 (6.6%) | 8 (53.3%) | 3 (20%) | 1 (6.6%) | 15 (100%) |
| Post-decontamination Kudoh | 0 (0%) | 1 (6.6%) | 5 (33.3%) | 8 (53.3%) | 1 (6.6%) | 15 (100%) |
| Post-decontamination modified Petroff | 0 (0%) | 0 (0%) | 2 (13.3%) | 10 (66.6%) | 3 (20%) | 15 (100%) |

**Table 2:** Comparison of contamination levels in Lowenstain-Jensen medium and 7H10 between Kudoh decontamination technique and Petroff modification

| | LJ (n total= 15) | | P-value | 7H10 (n total=15) | | P-value |
|---|---|---|---|---|---|---|
| | Kudoh n (%) | Modified Petroff n (%) | | Kudoh n (%) | Modified Petroff n (%) | |
| AFB (+) and non AFB (+) | 1 (6.6%) | 1 (6.6%) | | 1 (6.6%) | 1 (6.6%) | |
| Contamination of media | 1 (6.6%) | 0 (100%) | | 1 (6.6%) | 0 (100%) | |
| Total | 2 (13%) | 1 (6.6%) | 0.5 | 2 (13%) | 1 (6.6%) | 0.5 |

*Contamination rate*

The presence of contamination was performed by direct observation in the Lowenstein-Jensen, and the Middlebrook 7H10 medium, confirmed by ZN staining. In the Lowenstein-Jensen media using the Kudoh technique, 2 (13%) tubes were positive for contamination, whereas with the modified Petroff technique, 1 (6.6%) tube of media was positive for contamination. There were no significant differences between both methods. In the Middlebrook 7H10 media using the Kudoh technique, 2 (13%) tubes were positive for contamination, while with the modified Petroff technique, 1 (6.6%) tube was positive for contamination. There were no significant differences between both methods (Table 2).

*Recovery rate*

In the Kudoh technique, 13 (86.6%) showed positive results for MTB in the Lowenstein-Jensen media, whereas 14 (93.3%) with the modified Petroff technique. Colony growth of MTB in the Middlebrook 7H10 media using both methods showed positive results in each of 13 (86.6%) culture tubes.

In this study, the results of AFB staining from both decontamination techniques showed a sensitivity increase of 100% compared with before the decontamination process (86.7%). These are in concordance with a study conducted by Uddin *et al.* [3], which showed that the decontamination process could increase the sensitivity of microscopic AFB findings up to 12%, namely; 74% positive smear staining from direct specimens and 87% positive smear positive after the decontamination process [3]. Another study by Peterson in two different laboratory settings showed that smear staining from direct specimens was significantly less sensitive than after the decontamination process [13].

Acid-fast bacilli (AFB) microscopy is simple, inexpensive, and provides rapid results. However, AFB sputum staining from direct smear microscopy can only be detected if there are $10^4$-$10^5$ bacilli/mL sputum. The sensitivity and specificity of AFB microscopy are low when compared with the culture method [3].

As the Kudoh culture method does not require sample centrifugation, a reduced recovery rate of *M. Tuberculosis* would be a reasonable expectation. However, in this study, the Kudoh detection rate was similar to that of the modified Petroff culture. In addition, this minimal exposure did not affect the contamination rate. In our study, the contamination rate on the LJ by both methods was 2 (13%), while on the Middlebrook 7H10 by both methods showed 1 (6.6%).

Positive cultures with contamination were obtained in both mediums (13%) by the Kudoh and (6.6%) and by the modified Petroff technique. This study is in line with those conducted by Franco-Sotomayor and showed no significant difference in the contamination level between both methods [4]. Another study conducted by Costa further showed no significant difference in the level of contamination between the Kudoh technique and the Petroff modification, respectively 6.4% and 2.9% [2]. However, the results of this study are different from those of Jaspe *et al.* [14] showed that the contamination level in the Kudoh technique (3.7%) was significantly lower than in the Petroff technique (7.3%) [14]. In the Kudoh technique, the sample volume absorbed in the cotton swab is less than the sample used in the petroff modification technique, but the difference in sample volume has no effect on the recovery rate of MTB between both techniques when presented with NaOH. In the Kudoh technique, the exposure time to 4% short NaOH for 2 minutes is less deadly for MTB compared with the exposure time for NaOH for 15 minutes in the petroff modification technique, so that the petroff modification technique can produce lower positive culture, but this compensated with more sample volume (Sotomayor, 2020). Another study by Palaci *et al.* (2013) showed a low level of contamination with the Kudoh technique, at 3.8% if compared with NALC-NaOH, was 9.1% [10]. Research by Madeira also showed a lower level of contamination with the Kudoh technique compared with NALC-NaOH, which was 4.1% and 9%, respectively [12].

The positive culture results in Lowenstein-Jensen and 7H10 media between the Kudoh technique with modified Petroff did not show a statistically significant difference in the recovery rate. In the LJ media, the modified Petroff technique provides a better recovery rate than the Kudoh technique, which is 93.3% for the modified Petroff technique and 86.6% for the Kudoh technique. The recovery rate on the Middlebrook 7H10 with the modified Petroff technique provides the same recovery rate as the Kudoh technique, which is 86.6%.

A study by Palaci *et al.* (2013) reported that Kudoh technique had a sensitivity of 94.8% and a specificity of 99.8% in confirmed cases of culture in the LJ media using the NaLC-NaOH method [10]. Research conducted by Madeira *et al.* (2018) shows that Kudoh technique has a sensitivity of 80% and a specificity of 94% [11]. Another study by Costa *et al.* (2017) showed that Kudoh technique had a sensitivity of 90.4% and a specificity of 96.6% [2]. The recovery rate in the Kudoh technique (34.6%) was slightly better than the modified Petroff technique (37.1%), but statistically did not differ significantly in producing positive cultures [2]. In the sample Kudoh technique, it is presented at 4% NaOH for 2 minutes short incubation time for 2 minutes in a concentration of 4% NaOH is less deadly for MTB, so that it can maintain the MTB viability. In the Petroff modification technique, the specimen is exposed with 4% NaOH for 15 minutes, which was longer than the Kudoh technique, but the specimens volume is more than the Kudoh technique. NaOH is a decontaminant agent that can be toxic to mycobacteria. Therefore, decontamination with petroff modification techniques can produce lower positive culture, but this is compensated with more sample volume. This study's results align with research conducted by Franco-Sotomayor (2020). The recovery rate using the Kudoh method is 98.2%, while the Petroff technique is 96.6% [4].

In the Kudoh technique, it is estimated that about ± 250 l of the sample will be absorbed in the

cotton swab, much less than the sample used in the modified Petroff technique, which is 2 mL. However, the difference in sample volume does not significantly affect the recovery rate of MTB between the Kudoh technique compared with the modified Petroff when exposed to NaOH. The short incubation time of 2 minutes in a concentration of 4% NaOH is less lethal to MTB than the incubation time with NaOH for 15 minutes in the modified Petroff technique. Hence, the modified Petroff technique can produce lower positive cultures, but this is compensated by volume. A larger sample is 2 mL [4]. Mycobacteria cell walls contain high lipid content so that they are more resistant to acids and strong bases than other microorganisms, but too long exposure to NaOH can reduce mycobacteria viability by 60% [15].

In the Kudoh technique, it is estimated that about ± 250 l of the sample will be absorbed in the cotton swab, much less than the sample used in the modified Petroff technique, which is 2 mL. However, the difference in sample volume does not significantly affect the recovery rate of MTB between the Kudoh technique compared with the modified one. Petroff when exposed to NaOH. The short incubation time of 2 minutes in a concentration of 4% NaOH is less lethal to MTB than the incubation time with NaOH for 15 minutes in the modified Petroff technique, so the modified Petroff technique can produce lower positive cultures, but this is compensated by volume. A larger sample is 2 mL [4]. In this study, the positive culture results using the modified Petroff technique showed more colony growth than the Kudoh technique, indicating more viable MTB colonies than the Kudoh technique. This might be due to the Petroff modification technique; MTB initially is concentrated during the centrifugation process. Therefore, more MTB is inoculated into the culture media. Petroff modification technique shows the growth of the number of colonies more than the Kudoh technique. However, the Kudoh technique shows the results of positive culture that is no different to the level of recovery even though the number of colonies that grow in less Kudoh technique.

## Conclusion

Based on this study, it can be concluded that there was no difference in the contamination level in the cultures of Mycobacterium tuberculosis between Kudoh technique and modification Petroff. The Kudoh technique has the same effectiveness as the technique of Petroff modification.

## Disclosure Statement

No potential conflict of interest was reported by the authors.

## Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

## Authors' Contributions

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

## Orcid

*Andritta Febriana*
https://orcid.org/0000-0002-0334-1623
*Ni Made Mertaniasih*
https://orcid.org/0000-0002-0594-2385
*Pepy Dwi Endraswari*
https://orcid.org/0000-0002-0271-8505
*Lindawati Alimsardjono*
https://orcid.org/0000-0003-2771-4102
*Eko Budi Koendhori*
https://orcid.org/0000-0002-4412-0755

## References

[1]. World Health Organization. *TB disease burden. Dalam Global tuberculosis report 2020*; World Health Organization: Geneva, 2020 [Google Scholar], [Publisher]

[2]. da Costa R.R., da Silva S.F., Fochat R.C., Macedo R.L., Pereira T.V., Silva M.R., Pinto C.P.G.,

Leite I.C.G., Comparison between Ogawa-Kudoh and modified Petroff techniques for mycobacteria cultivation in the diagnosis of pulmonary tuberculosis, *Einstein (Sao Paulo)*, 2018, **16**:2 [Crossref], [Google Scholar], [Publisher]

[3]. Uddin M.K.M., Chowdhury M.R., Ahmed S., Rahman M.T., Khatun R., Leth F., Banu S., Comparison of direct versus concentrated smear microscopy in detection of pulmonary tuberculosis, *BMC research notes*, 2013, **6**:1 [Crossref], [Google Scholar], [Publisher]

[4]. Franco-Sotomayor Greta., Rivera-Olivero I.A., Leon-Benitez M., Uruchima-Campoverde S.E., Cardenas-Franco G., Perdomo-Castro M.E., Cardenas-Franco C.S., Ortega-Vivanco J., Abad-Ruiz A.S., de Waard J.H., Garcia-Bereguiain M.A., Fast, simple, and cheap: the Kudoh-Ogawa swab method as an alternative to the Petroff–Lowenstein-Jensen method for culturing of Mycobacterium tuberculosis, *Journal of Clinical Microbiology*, 2020, **58**:e01424 [Crossref], [Google Scholar], [Publisher]

[5]. Zijenah L.S., *The World health organization recommended TB diagnostic tools*, *Tuberculosis*, 2018, **2**:71 [Crossref], [Google Scholar], [Publisher]

[6]. Adam M.A.M., Ebraheem R.S.M., Berdri S.A., Statistical Investigation of High Culture Contamination Rates in Mycobacteriology Laboratory, *Frontiers in Microbiology*, 2022, **13**:151 [Crossref], [Google Scholar], [Publisher]

[7]. Tripathi K., Tripathi, P.C., Nema S., Shrivastava A. 'Modified Petroff's method: an excellent simplified decontamination technique in comparison with Petroff's method'. *International Journal of Recent in Science and Technology,* 2014*,* **8**:461 [Google Scholar], [Publisher]

[8]. Madeira C.M., Azam K.I., Sato D.N., Khosa C., Bhatt N., Viegas S.O. Evaluation of the Ogawa-Kudoh method for tuberculosis isolation in two health units in Mozambique. *African Journal of Laboratory Medicine*, 2020, **9**:929 [Google Scholar], [Publisher]

[9]. Palaci M., Peres R.L., Maia R., Cunha E.A., Ribeiro M.O., Lecco R., de Souza Ribeiro C., Ferro E Silva R.R., Vinhas S.A., Dietze R., Vianna S. & de Morais C.G. Contribution of the Ogawa-Kudoh swab culture method to the diagnosis of pulmonary tuberculosis in Brazil. *International Union Against Tuberculosis and Lung Disease*, 2013, **17**:782 [Crossref], [Google Scholar], [Publisher]

[10]. Palaci M., Peres R.L., Maia R., Cunha, E.A.T., Ribeiro M.O., Lecco R., de Souza Ribeiro C., Ferro E Silva R.R., Vinhas S.A., Dietze R., Vianna S., de Morais C.G.V., Contribution of the Ogawa-Kudoh swab culture method to the diagnosis of pulmonary tuberculosis in Brazil, *The International journal of tuberculosis and lung disease*, 2013, **17**:782 [Crossref], [Google Scholar], [Publisher]

[11]. Okeke I.N., African laboratory medicine in the time of COVID-19, *African journal of laboratory medicine*, 2020, **9**:1 [Crossref], [Google Scholar], [Publisher]

[12]. Rivas C., Coitinho C., Dafond V., Corbo M., & Baldjian M. Performance of the Ogawa-Kudoh method for isolation of mycobacteria in a laboratory with large-scale workload, *Revista Argentina de Microbiologia*, 2010, **42:**87 [Google Scholar], [Publisher]

[13]. Peterson E.M., Nakasone A., Platon-DeLeon J.M., Jang Y., de la Maza L.M., Desmond E., Comparison of direct and concentrated acid-fast smears to identify specimens culture positive for Mycobacterium spp, *Journal of clinical microbiology*, 1999, **37**:3564 [Crossref], [Google Scholar], [Publisher]

[14]. Jaspe R.C., Rojas YM., Flores L.A., Toro E.S., Takiff H., de Waard J.H., Evaluation of the Kudoh swab method for the culturing of Mycobacterium tuberculosis in rural areas, *Tropical Medicine & International Health*, 2009, **14**:468 [Crossref], [Google Scholar], [Publisher]

[15]. Chatterjee M., Bhattacharya S., Karak K., Dastidar S.G. Effects of different methods of decontamination for successful cultivation of Mycobacterium tuberculosis, *The Indian journal of Medical Research*, 2013, **138**:541 [Crossref], [Google Scholar], [Publisher]

**HOW TO CITE THIS ARTICLE**
Andritta Febriana, Ni Made Mertaniasih, Pepy Dwi Endraswari, Lindawati Alimsardjono, Eko Budi Koendhori. Effectiveness Comparison of Decontamination Sputum Specimen between Kudoh and Petroff Modification Technique in *Mycobacterium Culture*. *J. Med. Chem. Sci*., 2023, 6(9) 2096-2103
DOI: https://doi.org/10.26655/JMCHEMSCI.2023.9.16
URL: http://www.jmchemsci.com/article_169165.htmlV